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TITLE: Use of conductive sensors in diagnostic assays

ABPL:

A conductive sensor and its use in a diagnostic assay are disclosed. The miniaturized conductive sensor, utilizing a conducting polymer, is used in a diagnostic device to determine the presence or concentration of a predetermined analyte in a liquid test sample, wherein the predetermined analyte, like glucose, is assayed by an oxidase interaction. The interaction between the oxidase and a small amount of the predetermined analyte in the test sample generates, either directly or indirectly, a dopant compound in a reaction zone of the conductive sensor. The dopant compound then migrates to the detection zone of the conductive sensor of the diagnostic device to oxidize the conducting polymer and convert the conducting polymer from an insulating form to a conducting form. The resulting increase in conductivity of the conducting polymer is measured, then the conductivity increase is correlated to the concentration of the predetermined analyte in the test sample.

BSPR:

The present invention relates to a method of determining the presence or concentration of a predetermined analyte in a test sample with a diagnostic device including a miniaturized conductive sensor. The conductive sensor comprising a reaction zone including an enzyme that selectively interacts with the predetermined analyte and a detection zone including a layer or film of a conducting polymer and a microelectrode assembly. More particularly, a diagnostic device is employed to selectively assay a test sample for the presence or concentration of a specific predetermined analyte by measuring the change in conductivity of a layer of conducting polymer present in the detection zone of the conductive sensor. The conductivity change of the layer of conducting polymer results from the generation of a dopant compound in the reaction zone of the sensor due, either directly or indirectly, to an enzymatic interaction with the predetermined analyte. The dopant compound then migrates from the reaction zone of the sensor to the detection zone of the conductive sensor to dope the layer of conducting polymer and change the conductivity of the polymer. For example, a dopant compound, molecular iodine, is formed in the reaction zone in a reaction between iodide ions, a peroxidase enzyme or a molybdenum(VI) transition metal catalyst; and the hydrogen peroxide formed from a glucose oxidase interaction with glucose. The dopant compound then migrates, or diffuses, from the reaction zone to dope, oxidatively, the layer of conducting polymer in the detection zone. Surprisingly and unexpectedly, the configuration of the conductive sensor is such that only a small fraction of the glucose in the test sample, such as about 1% or less, is enzymatically converted to generate the dopant compound. Accordingly, oxygen limitations in the enzyme interaction are avoided. However, the amount of a generated dopant compound is sufficient to change the conductivity of the conducting polymer layer in the conductive sensor and allow an accurate conductivity measurement that can be correlated to the amount of the glucose in the test sample.

BSPR:

Investigators have therefore shown an intense interest in developing electrochemical sensors that can be miniaturized, and therefore either be

implanted subcutaneously or require a much smaller sample volume. Numerous approaches have been tried, including amperometric sensors based upon fiber electrodes and potentiometric sensors manufactured using techniques established in the semi-conductor industry. In contrast, the present invention measures a conductivity change in a thin polymer film, and accordingly has solved many of the problems inherent in miniaturized electrochemical sensors. Most importantly, an electrochemical sensor of the present invention can be manufactured in a planar format, using semi-conductor technologies, to provide an economical, disposable sensing element. Also, the sensor can be made sufficiently small such that a sample volume of 1 . μ L or less can be assayed. The sensitivity of the detection method also has solved the general oxygen limitation problem frequently observed in electrochemical sensors that utilize oxidase enzymes. Therefore, in accordance with an important feature of the present invention, an electrochemical sensor that can be miniaturized; that provides rapid and accurate assays; that overcomes oxygen limitation problems; that is free of interferences attributed to common constituents in the test sample; and that can be produced economically is achieved.

BSPR:

In general, a diagnostic device of the present invention comprises a reaction zone wherein the predetermined analyte of interest interacts with a suitable oxidase enzyme to generate, either directly or indirectly, a dopant compound. The dopant compound is capable of oxidizing a conducting polymer to change the conductivity of the polymer. In addition, the diagnostic device further comprises a detection zone including a film or layer of conducting polymer and a microelectrode assembly. The change in conductivity of the conducting polymer layer as a result of the dopant compound is detected or measured by the microelectrode assembly. The change in conductivity then is correlated to the amount of predetermined analyte in the test sample. As will be demonstrated more fully hereinafter, an economical and reproducible conductive sensor, useful in assaying for a predetermined analyte that responds to oxidase chemistry, has been provided. The conductive sensor utilizes the properties of conducting polymers, avoids oxygen limitation problems in the normal concentration ranges of the predetermined analyte; can be manufactured by well-known semiconductor processing techniques; and does not rely upon a chemical reaction occurring at the microelectrode assembly.

BSPR:

Accordingly, one important feature of the conductive sensor of the present invention is the conducting polymer included in the detection zone of the conductive sensor. Several organic conducting polymers, such as polyacetylene, polypyrrole and polythiophene, are known. The organic conducting polymers have several potential applications in the fields of batteries, display devices, corrosion prevention in metals and semiconductors and in microelectronic devices such as diodes, transistors, sensors, light emitting devices and energy conversion and storage elements. However, organic conducting polymers possess several limitations that have hindered the use of organic conducting polymers in conductive sensors. In general, a conducting polymer useful in the conductive sensor of the present invention should display a sufficiently high conductivity for detectable and accurate measurements, and should be capable of being processed, reproducibly, into thin, uniform films. Although several conducting polymers possess one of these two necessary properties, only a limited number of conducting polymers possess both necessary properties.

BSPR:

However, polyacetylene suffers from the drawbacks of extreme instability in air and a sharp drop in conductivity when an alkyl or other substituent group is introduced into the polymer. Accordingly, the instability of polyacetylene in the presence of oxygen, and the inability of substituted polyacetylenes to maintain a high conductivity, generally makes a polyacetylene unsuitable as the conducting polymer in a conductive analyte sensor. As will be discussed more fully hereinafter, conducting polymers including alkyl or other substituent groups usually possess physical and mechanical properties making the substituted conducting polymer more easily processible into conducting polymeric films than the corresponding unsubstituted conducting polymer.

Therefore, in the manufacture of conductive sensors it is desirable to use an easy-to-process conducting polymer, such as a conducting polymer that is stable in air and possesses suitable mechanical and physical properties, like solubility in organic solvents.

BSPR:

Similarly, the conducting polymer polypyrrole exhibits conductivities ranging from about 1 S/cm to about 100 S/cm. Investigators again found that placing substituent groups on either the nitrogen atom or a carbon atom of the heteroaromatic pyrrole ring decreases the conductivity of polypyrrole. For example, an unsubstituted polypyrrole, incorporating the tetrafluoroborate anion as the dopant compound, exhibits a conductivity of 40 S/cm, whereas the N-methyl derivative, incorporating the same dopant compound, exhibits a conductivity of 10×10^{-3} S/cm; the three-methyl derivative of pyrrole exhibits a conductivity of 4 S/cm; the 3,4-dimethyl derivative, a conductivity of 10 S/cm; and the 3,4-diphenyl derivative, a conductivity of 10×10^{-3} S/cm. Accordingly, substituted polypyrroles, although often demonstrating good physical properties, may not demonstrate a sufficient conductivity for use as the conducting polymer in a conductive analyte sensor.

BSPR:

As illustrated by the large conductivity drop in polypyrroles having substituents positioned on the pyrrole ring, even substituents as small as a methyl group introduce steric interactions sufficient to essentially destroy the conductivity of the polymer. However, as will be discussed more fully hereinafter, if a conducting polymer has a suitable substituent group present on the monomer units, reproducible processing of the conducting polymer into a thin film of uniform thickness is facilitated. Therefore, it would be desirable to provide a conductive sensor including a conducting polymer that is easy to process and that also exhibits a sufficiently high conductivity for sensitive and accurate analyte determinations.

BSPR:

Substituents placed on the heteroaromatic thiophene ring can affect the resulting conducting polymer. However, in contrast to pyrrole, ring substituents on thiophene do not seriously reduce the conductivity of the resulting heteroaromatic polymer. For example, it has been found that for 3-methylthiophene and 3,4-dimethylthiophene, the resulting substituted polythiophene exhibited an improved conductivity compared to the unsubstituted parent polythiophene, presumably due to enhanced order in the polymer chain of the substituted thiophene. Accordingly, unlike many pyrroles, substituents can be included on the thiophene monomer units to improve the processing properties of the resulting conducting polymer without adversely affecting the conductivity of the conducting polymer. Therefore, as a class, substituted polythiophenes are well-suited for use as the conducting polymers in a conductive analyte sensor.

BSPR:

From the studies on the polyacetylenes, polypyrroles and polythiophenes, and from related studies on other conducting polymers, it became apparent that a balance exists between the electronic effects and the steric effects introduced by the substituent on the monomer unit that renders a polymer of a substituted five or six member heteroaromatic ring more conducting or less conducting than the unsubstituted parent heteroaromatic compound. Therefore, it would be advantageous to utilize a conducting polymer having sufficient conductivity and suitable processing properties, such that the polymer can be used in a sensitive conductive analyte sensor of a diagnostic device to accurately determine the presence or concentration of a predetermined analyte in a liquid test sample.

BSPR:

Accordingly, the present invention is directed to a conductive sensor useful in a diagnostic assay for a predetermined analyte. More specifically, the conductive sensor is used in a diagnostic device to determine the presence or concentration of a predetermined analyte, like glucose, that is capable of

interacting with an oxidase enzyme. The predetermined analyte and the oxidase interact in a reaction zone of the conductive sensor to generate, either directly or indirectly, a dopant compound. The dopant compound then migrates to a detection zone of the conductive sensor to oxidatively dope a layer or film of conducting polymer, thereby altering the conductivity of the layer of conducting polymer. The change in conductivity of the conducting polymer then is detected or measured by a microelectrode assembly, and can be correlated to the concentration of the predetermined analyte in the test sample.

BSPR:

The prior art includes teachings related to diagnostic assays using conducting polymers. However, the known prior art does not include any references suggesting or anticipating the miniaturized conductive sensor of the present invention, or its method of use. Furthermore, although several references disclose the use of conducting organic polymers in sensors, no known prior art reference discloses a sensor demonstrating the sensitivity and accuracy of the conductive sensor of the present invention. The prior art sensors are based upon a direct interaction of an analyte, usually a gas, with the conducting polymer. In contrast, the present conductive sensors utilize a response to a dopant compound generated, either directly or indirectly, by an interaction between the predetermined analyte and an oxidase enzyme. The dopant compound then migrates, or diffuses, into the layer of conducting polymer and alters the conductivity of the layer of conducting polymer, thereby allowing the determination of the presence or concentration of the predetermined analyte in the test sample.

BSPR:

The observed sensitivity of the conducting polymer to the concentration of the dopant compound is important in the development of sensors that are based upon oxidase enzymes. As will be demonstrated more fully hereinafter, a very small amount of the dopant compound generates a response. Therefore, only a small fraction of the available predetermined analyte in the test sample, such as less than 1% of the available analyte, is interacted and converted into the dopant molecule. As a result, the problem of an oxygen limitation, inherent in using oxidase enzymes, is overcome, and a sensitive determination of the total analyte concentration in the test sample is achieved.

BSPR:

For example, M. K. Malmros et al., in the publication, "A Semiconductive Polymer Film Sensor for Glucose", Biosensors 3, pp. 71-87 (1987/88), suggest using polyacetylene in a biosensor to quantitatively detect glucose. Malmros et al. teach the conversion of glucose by glucose oxidase to gluconic acid and hydrogen peroxide, and the subsequent conversion of iodide ion to molecular iodine, or triiodide anion, by the action of lactoperoxidase. Malmros et al. further teach that the iodine so generated can be used to change the conductivity of a polyacetylene film, particularly as a modifier of the effect of the peroxide.

BSPR:

Wrighton et al., in European Patent Application Publication No. 185,941, disclose the use of conducting organic polymers as the active species in a chemical sensor. Wrighton et al. generally teach using the changes in physical properties of the conducting polymer as the active transduction into electrical signals. Specific examples cited in the patent include detection of oxygen gas, hydrogen gas, pH and enzyme substrate concentrations like glucose. The principal transduction mechanism described by Wrighton et al. is the direct use of the change in polymer conductivity induced by oxidation or by reduction. In contrast to the present invention, Wrighton et al. include the reaction catalyst, like an enzyme, in the conducting polymer matrix. Accordingly, the interaction occurs within the conducting polymer matrix. In the device and method of the present invention, the entire analyte-oxidase interaction occurs essentially in a reaction zone of the device to generate the dopant compound; the dopant compound then migrates from the reaction zone to a detection zone that includes the layer of conducting polymer. The rate of change of conductivity of the conducting polymer layer as the dopant compound

diffuses from the reaction zone to the detection zone is used to measure the concentration of the predetermined analyte in the test sample. Wrighton et al. do not teach a method of integrating the glucose oxidation by oxygen into the oxidation properties of the polymer, nor do Wrighton et al. teach the conversion of glucose to iodine through coupled enzymatic reactions. Furthermore, Wrighton et al. do not teach the use of solution processible polymers; all of the polymers used by Wrighton et al. are grown electrochemically.

BSPR:

Nagy et al., in the publication "Enzyme Electrode for Glucose Based on an Iodide Membrane Sensor," *Analytica Chim. Acta.*, 66, pp. 443-455 (1973), describe the detection of glucose by potentiometrically monitoring the disappearance of iodide ion. Nagy et al. monitor the decrease in iodide activity at the electrode surface, whereas the present invention monitors the amount of a predetermined analyte in the test sample by measuring the rate of change of conductivity of the conducting polymer due to the generation of a dopant compound by an oxidase enzyme mediated interaction.

BSPR:

In contrast, the method and device of the present invention utilizes a conductometric detection and measurement. Accordingly, an extremely low voltage can be used. The voltage is much lower than the oxidation potential of the interfering compounds, and therefore the interfering compounds are not oxidized. In addition, in the present invention, all chemical interactions occur in the reaction zone of the conductive sensor. The molecular iodine is generated in the reaction zone and migrates to dope the conducting polymer in the detection zone. Therefore, no direct interference is possible at the electrode.

BSPR:

However, the generated molecular iodine dopant compound is capable of interacting with various serum components, like ascorbate. If a sufficient amount of the molecular iodine interacts with serum components rather than doping the polymer, interferences are observed. Therefore, the method of the present invention relies upon fast assays to minimize interfering reactions of the generated molecular iodine. This is accomplished by the configuration of the sensor of the present invention, comprising a thin reaction zone and a thin detection zone, such that the molecular iodine is generated near the conducting polymer to quickly dope the conducting polymer before significant interfering reactions can occur. Furthermore, in the preferred embodiment of the present invention, it will be demonstrated that a semipermeable membrane utilized to meter the test sample into the reaction zone also selectively screens interfering compounds from the test sample, and therefore precluding an interaction with the generated molecular iodine.

BSPR:

Therefore, the method of the present invention allows the accurate assay of a predetermined analyte that is responsive to oxidase chemistry. The method utilizes a diagnostic device that includes a conductive sensor, wherein the conductive sensor comprises a reaction zone and a detection zone. The reaction zone of the conductive sensor is a thin film including the reagents necessary to interact with the predetermined analyte and to generate a dopant compound. The detection zone includes a film or layer of a conducting polymer and a microelectrode assembly such that the dopant compound can migrate to the detection zone to dope the conducting polymer, and such that the resulting change in conductivity, detected and measured by the microelectrode assembly, can be correlated to the amount of predetermined analyte in the test sample. The conductive sensor overcomes the disadvantages demonstrated by the prior art sensors, and therefore provides sensitive, accurate and reproducible assays; provides a fast assay, such as within 30 seconds, and preferably within 10 seconds, from a small blood sample, such as from about 0.1 μl to about 5 μl ; eliminates the oxygen limitation problem associated with oxidase chemistry; eliminates the problems associated with interfering compounds present in the test sample; demonstrates excellent shelf stability;

is economical and disposable; and is miniaturized and can be reproducibly manufactured by semiconductor processing techniques.

BSPR:

In brief, the present invention is directed to a diagnostic device including a conductive analyte sensor comprising a reaction zone and a detection zone, wherein the detection zone includes a conducting polymer and a microelectrode assembly. More particularly, the present invention is directed to a conductive sensor that allows the sensitive and accurate detection and measurement of a predetermined analyte in a liquid test sample, wherein the predetermined analyte is assayed by an oxidase interaction. In accordance with the method of the present invention, an interaction between the predetermined analyte and an oxidase enzyme occurs in the reaction zone of the conductive sensor to produce, either directly or indirectly, a dopant compound that migrates to the detection zone of the sensor. The detection zone of the device is in laminar contact with the reaction zone and includes a layer or film of conducting polymer that is oxidized by the dopant compound. Therefore, the conductivity of the conducting polymer layer is changed, and the change in conductivity of the conducting polymer layer is detected and measured by the microelectrode assembly and is correlated to the concentration of the predetermined analyte in the test sample.

BSPR:

The conductive sensors of the present invention utilize the unique electrical properties of conducting polymers to determine the presence and concentration of a predetermined analyte that is capable of interacting with a specific oxidase enzyme. In accordance with the method and device of the present invention, the conductive sensors include a layer of conducting polymer in a detection zone of the sensor. The conducting polymer is oxidized by a dopant compound generated, either directly or indirectly, as a result of an interaction between the predetermined analyte and an oxidase enzyme in a reaction zone of the sensor. The dopant compound migrates from the reaction zone to the detection zone of the device to oxidize the layer of conducting polymer. The conductivity of the polymer, therefore, is changed by the introduction of the dopant compound into the conducting polymer layer, and the measurable conductivity change is detected and measured by a microelectrode assembly in the detection zone and is correlated to the concentration of the predetermined analyte in the test sample.

BSPR:

Consequently, it has been demonstrated that the conductive sensor of the present invention allows an accurate and sensitive electrical transduction of an analyte-oxidase interaction, like a glucose-glucose oxidase interaction. In accordance with an important feature of the present invention, the sensitive and accurate detection of a predetermined analyte, like glucose, results from the effect of the ultimately generated dopant compound, like molecular iodine, upon the conductivity of a conducting polymer layer. This particular type of reaction and detection method is known in the art. However, to date, utilizing this reaction in a conductive sensor has not provided a sensitive and accurate assay for a predetermined analyte.

BSPR:

Therefore, and in accordance with the present invention, an interaction between a predetermined analyte and an oxidase enzyme, and a subsequent interaction between the generated hydrogen peroxide, a dopant compound precursor and a peroxidase enzyme or a compound that exhibits peroxidase activity to generate a dopant compound, like molecular iodine, occurs in a reaction zone of a conductive sensor of a diagnostic device. The dopant compound migrates from the reaction zone to contact and oxidatively dope a layer or film of conducting polymer present in a detection zone of the sensor. Consequently, the conductivity of the conducting polymer layer is changed, and the concentration of the predetermined analyte is determined from the change in conductivity of the conducting polymer by a microelectrode assembly in the detection layer. Thus, to provide an accurate and sensitive assay by eliminating oxygen limitation problems in the oxidase-based reaction, the

change in conductivity of the conducting polymer layer in the conductive sensor is measured within about thirty seconds, and preferably within about 15 seconds, after the test sample contacts the reaction zone of the conductive sensor. To achieve the full advantage of the present invention, the change in conductivity is measured from about 5 seconds to about 10 seconds after the test sample contacts the reaction zone of the sensor.

BSPR:

Therefore, it is an object of the present invention to provide a method of determining the presence or concentration of a predetermined analyte in a liquid test sample by utilizing a diagnostic device including a conductive sensor comprising a reaction zone in contact with a detection zone. It also is an object of the present invention to provide a method of determining the concentration of a predetermined analyte in a liquid test sample wherein the predetermined analyte interacts with an oxidase enzyme in the reaction zone of the sensor to generate, either directly or indirectly, a dopant compound that oxidizes a layer or film of conducting polymer in the detection zone of the sensor and that changes the conductivity of the conducting polymer.

BSPR:

Another object of the present invention is to provide a method of determining the presence or concentration of a predetermined analyte in a liquid sample comprising contacting a diagnostic test device with the liquid sample, wherein the diagnostic test device includes a conductive sensor comprising a reaction zone, wherein a portion of the predetermined analyte interacts with an oxidase enzyme and other reagents, if necessary, to generate a dopant compound, and a detection zone in contact with the reagent zone, such that the dopant compound migrates to the detection zone to dope a layer of conducting polymer present in the detection zone and to cause a detectable or measurable change in the conductivity of the conducting polymer; measuring the change in conductivity of the layer of conducting polymer by a microelectrode assembly present in the detection zone; and correlating the change in conductivity of the layer of conducting polymer to the concentration of the predetermined analyte in the test sample.

BSPR:

Another object of the present invention is to provide a sensitive conductive sensor that accurately senses the presence or concentration of a predetermined analyte in a liquid test sample, wherein the predetermined analyte is capable of interacting with an oxidase enzyme, comprising: a) a reaction zone including a hydratable host matrix permeable to the predetermined analyte and having homogeneously incorporated therein a suitable oxidase enzyme, a peroxidase enzyme or a compound that exhibits peroxidase activity, and any other necessary reagents, like a dopant compound precursor, to generate a dopant compound, and wherein the predetermined analyte interacts with the oxidase enzyme, peroxidase and other reagents, if present, to generate, either directly or indirectly, the dopant compound; b) a detection zone in contact with the reaction zone including a layer or film of a conducting polymer in contact with a microelectrode assembly, such that the dopant compound generated in the reaction zone can migrate to and oxidatively dope the film or layer of the conducting polymer; and c) means operatively connected to the microelectrode assembly of the detection zone for measuring the conductivity of the conducting polymer.

BSPR:

Another object of the present invention is to provide a sensitive conductive sensor that accurately senses the presence or concentration of a predetermined analyte in a liquid test sample, wherein the predetermined analyte is capable of interacting with an oxidase enzyme, comprising: a) semipermeable membrane capable of effectively separating the cellular material and interfering components from the test sample while allowing the predetermined analyte to diffuse through the semipermeable membrane; b) a reaction zone including a hydratable host matrix permeable to the predetermined analyte and having homogeneously incorporated therein a suitable oxidase enzyme, a peroxidase enzyme or a compound capable of exhibiting peroxidase activity, and any other

necessary reagents, like a dopant compound precursor, to generate a dopant compound, and wherein the predetermined analyte interacts with the oxidase enzyme, the peroxidase enzyme or a compound that exhibits peroxidase activity, and other reagents, if present, to generate, either directly or indirectly, the dopant compound; c) a detection zone in contact with the reaction zone including a layer or film of a conducting polymer in contact with a microelectrode assembly, such that the dopant compound generated in the reaction zone can migrate to and oxidatively dope the film or layer of the conducting polymer; and d) means operatively connected to the microelectrode assembly of the detection zone for measuring the conductivity of the conducting polymer.

BSPR:

Another object of the present invention is to provide an accurate and sensitive miniaturized conductive sensor for determining the presence or concentration of glucose in a liquid test sample of less than 1 .mu.L comprising a reaction zone wherein the hydratable host matrix is a polymer matrix, such as a gelatin matrix or a chitosan matrix, incorporating therein glucose oxidase, peroxidase and iodide ion to interact with the glucose and to generate molecular iodine as the dopant compound upon contact between the liquid test sample and the reaction zone.

BSPR:

Another object of the present invention is to provide an accurate and sensitive miniaturized conductive sensor for determining the presence or concentration of a predetermined analyte capable of interacting with an oxidase enzyme comprising a detection zone comprising a layer, such as a thin film, of a conducting polymer in contact with a microelectrode assembly capable of measuring resistances as high as about 10.sup.9 ohms.

DRPR:

FIG. 1 is a partial side view in cross-section of a diagnostic device of the present invention comprising a capillary for introducing the test sample; and a conductive sensor comprising a reaction zone for interacting the predetermined analyte with an oxidase enzyme and for generating the dopant compound, and a detection zone for detecting the amount of dopant compound generated in the reaction zone by measuring the change in conductivity of a layer of conducting polymer present in the detection zone with a microelectrode assembly;

DRPR:

FIG. 5 is a plot of current (microamps at 10 sec.) vs. glucose concentration (mg/dL) showing the linear relationship between the concentration of glucose in a standardized test sample and conductivity of the layer of conducting polymer exhibited by a conductive sensor of the present invention;

DRPR:

FIG. 7 is a plot of current (amps at 10 seconds) vs. glucose concentration (mg/dL) showing the linear relationship between the concentration of glucose in a standardized test sample and conductivity of the layer of conducting polymer exhibited by a conductive sensor of the present invention;

DRPR:

FIG. 9 is a plot of current (microamps at 10 sec.) vs. glucose concentration (mg/dL) showing the linear relationship between the concentration of glucose in a standardized test sample and conductivity of the layer of conducting polymer exhibited by a conductive sensor of the present invention; and

DEPR:

In accordance with the method and device of the present invention, a diagnostic device includes a conductive sensor comprising a reaction zone and a detection zone, wherein the detection zone includes a layer of a conducting polymer and a microelectrode assembly, to determine the presence or concentration of a predetermined analyte in a liquid test sample. Although conductive sensors have been studied extensively, the use of a conductive

sensor in a diagnostic device to assay for a predetermined analyte, like glucose, has been impeded by several problems, including poor conducting polymer properties, impractical methods of manufacturing the conductive sensor, the inability to accurately test for a predetermined analyte, insensitivity to low concentrations of the predetermined analyte, irreproducible analyte assays, interferences associated with compounds often found in test samples, and oxygen limitation problems in assays based on oxidase chemistry. As will be described more fully hereinafter, the method and device of the present invention surprisingly and unexpectedly overcome many of the problems previous investigators encountered in attempts to incorporate a miniaturized conductive sensor into a diagnostic device.

DEPR:

Accordingly, the method and device of the present invention are based upon the oxidative doping of a conducting polymer, like a poly(3-alkylthiophene), present in the detection zone of the conductive sensor, by a dopant compound, such as molecular iodine, that is generated in the reaction zone of the sensor. As will be demonstrated more fully hereinafter, a poly(3-alkylthiophene), as depicted in structural formulas (I) (undoped) and (II) (doped), is a particularly useful conducting polymer in the method and device of the present invention. However, it should be understood that other conducting polymers demonstrating sufficient conductivity, i.e. detectable or measureable, and having sufficient stability, mechanical properties and processibility also are useful in the device and method of the present invention. For example, another class of conducting polymers exhibiting properties useful in the device and method of the present invention includes the poly(thienylene vinylene) polymers, depicted in general structural formula (III), and the related poly(furylene vinylene) polymers. A poly(thienylene vinylene) film doped with molecular iodine exhibited a conductivity of 62 S/cm, and a poly(furylene vinylene) film doped with molecular iodine exhibited a conductivity of 36 S/cm. ##STR1##

DEPR:

As previously discussed, the oxidized form of a conducting polymer, i.e. structure (II), demonstrates a dramatic increase in electrical conductivity compared to the reduced form of the conducting polymer, i.e. structure (I). For example, a conducting polymer in its completely reduced form is insulating, and exhibits a conductivity of about 10^{-7} S/cm. However, if the conducting polymer is fully oxidized, the conductivity increases to as high as about 10^{+3} S/cm, depending upon the chemical structure of the conducting polymer and the morphological condition of the conducting polymer layer. Furthermore, as disclosed by Burks and Hodge, in J. Chem. Phys., 83, p. 5796 (1985), a conducting polymer requires approximately 10^{-11} moles of dopant compound per square centimeter of surface area of conducting polymer to demonstrate an order of magnitude change in the conductivity of a 200.Å. (Angstrom) thick film of conducting polymer. Accordingly, this very sensitive conductivity response of the conducting polymer, especially a thin film of conducting polymer, to the concentration of the dopant compound doping the conducting polymer layer allows a conductive sensor of the present invention to provide sensitive and accurate assays for a predetermined analyte.

DEPR:

The increased conductivity of a conducting polymer upon oxidative doping by a dopant compound, such as molecular iodine or arsenic trifluoride, is a well recognized electrical property of a conducting polymer. However, it has been difficult to provide a uniform, thin film of a conducting polymer. If a thin film of polymer is available, less dopant compound is needed to provide a detectable or measurable increase in conductivity. Surprisingly and unexpectedly, the conductive sensor and the method of the present invention provide a uniform, thin film of conducting polymer, such as from about 100.Å. to about 1500.Å. in thickness. Therefore, the sensitivity of the assay is increased because less dopant compound must be generated for a detectable response; and the accuracy of the assay is increased because the electrical response of a layer of the conducting polymer is increased, therefore making detection measurements easier and more reliable. In addition,

and as will be demonstrated more fully hereinafter, the ability to provide a thin, uniform layer of a conducting polymer helps eliminate the oxygen limitation problem found in oxidase-based assays. The sensitive detection provided by a thin film of conducting polymer allows a method wherein only a very minor portion of the analyte in the test sample interacts with the enzyme and the available oxygen. Without the sensitivity provided by a thin film of conducting polymer, the change in conductivity provided by such a small conversion of analyte could go undetected.

DEPR:

Therefore, in accordance with the device and method of the present invention, a sufficient amount of ambient oxygen is present in the reaction zone 14 to allow a sufficient amount of the glucose in the test sample to interact with the oxidase enzyme and generate a sufficient amount of molecular iodine to dope a layer or film of conducting polymer 16 present in a detection zone of the conductive sensor of test device 10 and

DEPR:

cause a detectable or measurable conductivity change in the layer or film of conducting polymer 16 (FIG. 1c). Surprisingly and unexpectedly, the method and device of the present invention provide a conductive sensor that is sufficiently sensitive and operates sufficiently quickly such that low concentrations of generated molecular iodine are detected. Therefore, it is not necessary to convert the entire amount of glucose in the test sample to molecular iodine. Accordingly, the sensitivity of the conductive sensor, in addition to a configuration that allows a conductivity measurement before the oxygen supply is depleted, overcomes the oxygen limitation found in prior art methods and devices.

DEPR:

The molecular iodine produced in the reaction zone 14 migrates to a detection zone of the conductive sensor that is in contact with reaction zone 14 (FIG. 1c). The detection zone includes a film or layer of conducting polymer 16 and a microelectrode assembly 20. The top surface of the film of conducting polymer 16 is in contact with the reaction zone 14, and the bottom surface of the conducting polymer 16 is in contact with the microelectrode assembly 20. In general, in the detection zone, the molecular iodine oxidatively dopes the film or layer of conducting polymer 16 and the conductivity of the film or layer of conducting polymer 16 increases. A conductometric measurement is made under a constant applied potential between two microelectrodes present in the microelectrode assembly 20 of the detection zone, and the increase, or the rate of increase, of the conductivity of the film or layer conducting polymer 16 is correlated to the glucose concentration of the test sample. To achieve the full advantage of the present invention, the rate of increase in conductivity is measured before the supply of ambient oxygen is significantly depleted, such as from within 5 seconds to 30 seconds, and preferably from within 5 seconds to 10 seconds, after the test sample contacts the reaction zone 14 of the conductive sensor of test device 10.

DEPR:

In particular, the microelectrode assembly 30 illustrated in FIG. 2 comprises a base 32 of a smooth, nonconductive material, like silicon metal, ceramic or glass. The base 32 has interdigitated patterns of a conductive material 34 and 36, like a metal, applied to the top surface of the base 32. The interdigitated patterns of conductive material 34 and 36 are conductively connected to conductive contacting pads (not shown) on the bottom surface of the base 32 by conductive vias (not shown) incorporated into the base 32. The interdigitated patterns of conductive material 34 and 36 serve as the microelectrodes of the microelectrode assembly of the conductive sensor. Therefore, when a layer or film of conducting polymer is applied to the microelectrode assembly 30, the film or layer of conducting polymer bridges, or fills, the gap 38 between the interdigitated patterns of conductive material 34 and 36 and a change in conductivity of the layer or film of conducting polymer in the gap 38 is detected by the microelectrodes comprising the interdigitated patterns of conductive material 34 and 36. The manufacture of a microelectrode assembly 30

will be discussed more fully hereinafter.

DEPR:

In further regard to FIG. 1, in addition to the microelectrode assembly 20, another essential component in the detection zone of the conductive sensor is a thin, uniform layer or film of conducting polymer 16. The conducting polymer included in the layer or film of conducting polymer 16 usually is a heteroaromatic conducting polymer, like a polypyrrole, a poly(thienylene vinylene), a poly(furylene vinylene), a polyfuran or a polythiophene. However, a carbocyclic aromatic conducting polymer, like polyaniline, also is envisioned as useful in the method and device of the present invention. As will be discussed more fully hereinafter, a substituted polythiophene is the preferred conducting polymer because the substituted polythiophenes possess sufficient electrical properties and physical properties, like solubility in organic solvents, for a sensitive and accurate detection of the dopant compound and for easy and uniform manufacture of a thin film of conducting polymer.

DEPR:

The choice of conducting polymer used in the film or layer of conducting polymer 16 also is important because the conducting polymer preferably is easily processible when in solution, and exhibits rapid, facile oxidative doping upon exposure to a suitable dopant compound. A particularly useful class of polymers is the poly(3-alkylthiophene) polymers. Polyalkylthiophenes including an alkyl substituent with at least four carbon atoms exhibit significant solubility in many organic solvents including chloroform, methylene chloride, xylene and tetrahydrofuran. This solubility in organic solvents permits the use of various processing techniques that reliably and uniformly deposit thin polymer layers, or films, onto a substrate. These processing techniques include batch process techniques like spin coating, film casting, ink jet printing and similar batch process techniques. In particular, spin coating is a preferred method of depositing a layer or film of the conducting polymer onto a substrate electronic template. Similarly, polyaniline compounds can be solubilized in concentrated sulfuric acid and cast as a thin uniform film; and poly(thienylene vinylene) and poly(furylene vinylene) prepolymers are soluble and processible in the pre-polymer stage and can be cast, then polymerized. Although each of these types of polymers can be processed into thin films, the processing is relatively difficult. Accordingly, polymers such as the polyalkylthiophenes that are soluble in common organic solvents for simple casting into a film are preferred. In addition, as will be demonstrated more fully hereinafter, the reagents and the hydratable host matrix comprising the reaction zone 14 can be formed into a uniform, thin layer by the same batch-type processes used to form a thin layer of conducting polymer.

DEPR:

The morphological and electrical properties of the film or layer of conducting polymer included in the conductive sensor also can be modified by incorporating a surfactant or an inert, nonconducting polymer, such as, but not limited to, polyethylmethacrylate, polyacrylonitrile, polyethylene oxide, polyvinylidene chloride, nylon, polystyrene, polyacrylic acid, polyacrylamide, polyester, and similar nonconducting polymers, into the layer or film of conducting polymer. For example, an inert, nonconducting polymer, such as polymethylmethacrylate (PMMA), can be solubilized in an organic solvent, like chloroform, with a conducting polymer to form a casting solution. If the conducting polymer is included in the casting solution in a concentration sufficient to ensure electrical percolation, i.e. at least about 25% poly(3-octylthiophene) for a poly(3-octylthiophene)/PMMA casting solution, the final conductivity of the film of conducting polymer after oxidative doping by molecular iodine is approximately equal to the conductivity demonstrated by a doped 100% 3-alkylthiophene polymer film.

DEPR:

In addition to the particular chemical and physical properties required of the film or layer of conducting polymer 16 and the microelectrode assembly 20 in

the detection zone of the conductive sensor of the test device 10 in FIG. 1, the reaction zone 14 also should possess suitable chemical and physical properties for the device and method of the present invention to detect and accurately measure the predetermined analyte in the test sample. In general, the reaction zone 14 of the conductive sensor of test device 10 illustrated in FIG. 1 is a layer from about 0.1.mu. to about 10.mu., and preferably from about 0.2.mu. to about 5.mu., in thickness, when dry. To achieve the full advantage of the present invention, the reaction zone is a layer from about 0.2.mu. to about 3.mu. in thickness, when dry. However, the thickness of the layer of hydratable host matrix is limited only in that the layer is sufficiently thick to incorporate the necessary amounts of the oxidase enzyme, peroxidase and dopant compound precursor; and is sufficiently thin such that the assay can be performed within 30 seconds without interference from other compounds often found in a test sample. If the hydratable host matrix is too thick, the molecular iodine is generated relatively far from the layer of conducting polymer, therefore requiring more time for the molecular iodine to migrate to the layer of conducting polymer and providing time for the generated molecular iodine to interact with interfering compounds in the test sample, like ascorbate ion, thereby providing an inaccurate assay.

DEPR:

In accordance with an important feature of the present invention, the dry, hydratable host matrix is capable of rapid hydration upon contact with the liquid test sample, such as within about 5 seconds. In particular, the method of the present invention utilizes an early conductivity measurement, i.e. within about 30 seconds, and preferably within about 10 seconds, after the test sample contacts the reaction zone 14, to determine the rate of molecular iodine formation, or equivalently, the rate of glucose conversion. Therefore, it is important that the dry, hydratable host matrix hydrates before a substantial interaction between the predetermined analyte and the oxidase enzyme occurs. It also is important that the diffusion rates of all interactants and interaction products, like molecular iodine, through the hydratable host matrix are sufficiently high such that the interactions can proceed quickly, and that the generated molecular iodine can quickly and effectively migrate to the detection zone of conductive sensor to dope the layer or film of conducting polymer film 16.

DEPR:

Accordingly, the method of the present invention comprises introducing a test sample, such as from about 0.1 .mu.L to about 5 .mu.L, and usually less than 1 .mu.L, of a whole blood sample, into the capillary tube 12 of test device 10 illustrated in FIG. 1; then determining the change of conductivity in the layer of conducting polymer 16 in the detection layer within a time period of from about 5 sec. to about 30 sec. after the test sample contacts the reaction zone 14 of the conductive sensor of the test device 10. The change in conductivity of the layer or film of conducting polymer 16 is measured by the microelectrode assembly 20 and can be correlated to the amount of predetermined analyte in the test sample by comparison to the change in conductivity of a layer or film of conducting polymer 16 exhibited by standardized solutions of the predetermined analyte. Accordingly, a fast, simple, sensitive and accurate assay for glucose, or other analytes capable of interacting with an oxidase enzyme, is provided.

DEPR:

The preferred embodiment of the present invention is illustrated in FIG. 3, wherein a conductive sensor 40 is utilized to assay for a predetermined analyte, such as glucose, in a test sample 48. The conductive sensor 40, like the conductive sensor in the test device 10 illustrated in FIG. 1, includes a reaction zone 44 in laminar contact with a detection zone including a film or layer of a conducting polymer 46 and a microelectrode assembly 50 that are essentially identical to the reaction zone 14 and the detection zone of the conductive sensor of test device 10 of FIG. 1 described above. For example, reaction zone 44 includes the necessary oxidase enzyme to interact with the predetermined analyte of interest, peroxidase and a dopant compound precursor, if necessary, to generate a dopant compound. However, unlike the conductive

sensor of test device 10 in FIG. 1, the reaction zone 44 of conductive sensor 40 includes an excess amount of oxidase enzyme and peroxidase to convert a sufficient amount of the predetermined analyte in the test sample 48 to the dopant compound. In addition, the detection zone includes a layer or a film of conducting polymer 46 that is oxidatively doped by the dopant compound generated in reaction zone 44 and that migrates into detection zone.

DEPR:

However, whereas the test device 10 of FIG. 1 relied upon measuring the initial rate of interaction between the predetermined analyte and the oxidase enzyme included in reaction zone 14, the conductive sensor 40 illustrated in FIG. 3 controls the amount of the dopant compound that migrates into the detection zone from reaction zone 44 by limiting the migration of the test sample 48 into the reaction zone 44 by a semipermeable membrane 42. By limiting the amount of the test sample 48, and therefore glucose, migrating through the restrictive semipermeable membrane 42 to reaction zone 44, and by interacting and converting substantially all of the glucose migrating into reaction zone 44 to generate a dopant compound, the amount of glucose in the test sample 48 is determined by correlating the rate of change in conductivity of the layer or film of conducting polymer 46 in the detection zone to the diffusion rates of the test sample 48 through the semipermeable membrane 42.

DEPR:

The dopant compound, like molecular iodine generated in the reaction zone 44, then migrates to the detection layer to oxidatively dope the layer or film of conducting polymer 46 present in the detection zone of the conductive sensor 40. Accordingly, the layer or film of conducting polymer 46 exhibits an increase in conductivity, with the increase in conductivity being related to the migration rate of the predetermined analyte through semipermeable membrane 42. The increase in conductivity is detected by a microelectrode assembly 50 that is in contact with the layer or film of conducting polymer 46. Therefore, the concentration of the predetermined analyte is determined indirectly from the migration rate of the predetermined analyte in the test sample 48 through the semipermeable membrane 42.

DEPR:

Therefore, onto the top sensing surface of the microelectrode assembly is positioned a layer or film of a suitable conducting polymer. As stated previously, a suitable conducting polymer provides a resistance upon exposure to molecular iodine in the range from about 10.sup.1 ohm to about 10.sup.9 ohm when the polymer film has a thickness of from about 100.ANG. to 1000.ANG.. Furthermore, the conducting polymer should be stable and easy to process such that the conducting polymer can be applied to the microelectrode assembly as a uniform, thin film or layer by spin coating, film casting, jet printing or a similar application technique known in the art. The thickness of the film or layer of the conducting polymer is sufficient to fill the gaps between the two isolated interdigitated patterns on the substrate electronic template and to completely coat, or cover, the interdigitated patterns such that the interdigitated patterns of conducting material do not contact the reaction zone of the conductive sensor.

DEPR:

Therefore, in the manufacture of a conductive sensor 40 of FIG. 3, a particularly useful conducting polymer, poly(3-octylthiophene), first was dissolved in a suitable solvent, like xylene, at a concentration of 5 mg (milligrams) of conducting polymer per ml (milliliter) of solvent. In general, it has been found that the concentration of the conducting polymer in the solvent should range from about 2 mg/ml to about 15 mg/ml, and preferably from about 3 mg/ml to about 10 mg/ml. More concentrated solutions of the conducting polymer also can be used as long as the viscosity of the solution is suitable for casting a uniform, thin film of conducting polymer.

DEPR:

Then, positioned in laminar contact with the top surface of the reaction zone, a thin film of a silicone elastomer was applied to the conductive sensor. The

silicone elastomer served as the semipermeable membrane. The semipermeable membrane was applied to the conductive sensor by the same batch type processes described above to provide, reproducibly, a thin, uniform film of silicone elastomer having a thickness of about 6.mu. to about 8.mu.. It should be understood that for each of the three layers or films, i.e. the conducting polymer, the reaction zone and the semipermeable membrane, once the desired thickness of the film is determined, the semiconductor processing techniques utilized to manufacture the conductive sensors provide layers or films that are reproducible to within $\pm .5\%$ in thickness. It is important that the thickness of each layer be controlled such that the thickness of the individual layers is essentially eliminated as a variable parameter in the assay for a predetermined analyte. Therefore, in accordance with an important feature of the present invention, the thickness of each layer is controlled to within $\pm .5\%$ of the desired, predetermined thickness.

DEPR:

In addition to allowing the easy and economical manufacture of disposable test devices that require very small sample sizes, the ability to reproducibly cast uniform thin films provides a fast and accurate assay for a predetermined analyte. The thin films allow a sufficient amount of the predetermined analyte to migrate through the semipermeable membrane to contact the reaction zone and interact with the enzymes and the dopant compound precursor. After interacting to generate the dopant compound, the conducting polymer then is doped to provide an assay within about 30 seconds, and preferably within about 10 seconds. In addition, the assay is accurate and sensitive because the films or layers are sufficiently thin such that the dopant compound is generated in proximity to the conducting polymer layer. Accordingly, the dopant compound can dope the conducting polymer before undergoing reactions with interferent compounds often present in the test sample. Therefore, essentially all the dopant compound is available to dope the polymer to provide an accurate assay. Furthermore, because the conducting polymer is thin, the sensitivity of the conductive sensor is increased because a relatively large change in conductivity is observed for a relatively small amount of generated dopant compound.

DEPR:

The ability to reproducibly provide thin uniform films also is important because only a small portion of the total available predetermined analyte in the test sample is interacted to generate the dopant compound. Therefore, to accurately correlate the change in conductivity of the conducting polymer to the total amount of predetermined analyte in the test sample, the layers in the conductive sensor are manufactured at a reproducible thickness such that assay measurements are indicative of the actual concentration of predetermined analyte in the test sample, and not of the apparent concentration of predetermined analyte in the test sample because of appreciable thickness variances in any of the three films or layers in the sensor.

DEPR:

In accordance with an important feature of the present invention, a test device prepared by the above described method, but lacking the semipermeable membrane, i.e. a test device 10 of FIG. 1, accurately assayed standardized glucose solutions including from 25 mg/dL to 500 mg/dL glucose. The standardized glucose solutions further included iodide ions. As discussed above, in a test device of the present invention however, the iodide ions are included in the reaction zone of the conductive sensor in an amount ranging from about 30 mM to about 500 mM. The conductivity of the film or layer of conducting polymer in the detection zone was measured at an applied voltage of 0.1 V (volt), and electrical currents the range of from 0.1 .mu.amp (microamp) to 5 .mu.amp were found as typical.

DEPR:

In accordance with another important feature of the present invention, another test device was prepared by the above-described method and included a semipermeable membrane, i.e. a test device 40 of FIG. 3. This test device accurately assayed standardized glucose solutions including from 5 mg/dL to

500 mg/dL glucose. The standardized glucose solutions further included iodide ions. As discussed above and illustrated below, in a test device of the present invention, the iodide ions preferably are included in the reaction zone of the conductive sensor in an amount ranging from about 50 mM to about 500 mM. The conductivity of the film or layer of conducting polymer in the detection zone was measured at an applied voltage of 0.1 V (volt), and electrical currents the range of from 0.1 .mu.amp (microamp) to 5 .mu.amp were found as typical.

DEPR:

For example, it is envisioned that a test device of the present invention is an economical, miniaturized, disposable device. Each component in every embodiment of the present invention can be manufactured in a batch processing technique. In addition, the microelectrode assembly can be manufactured by a number of techniques well-known in the art utilizing a silicon, ceramic, glass or plastic base. Furthermore, the conductive sensor is stable because the method and device utilize an undoped, reduced layer conducting polymer, as opposed to a less stable, oxidized conducting polymer utilized in most prior art conductive sensors.